

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</p>					
1. REPORT DATE (DD-MM-YYYY)		2. REPORT TYPE		3. DATES COVERED (From - To)	
09/24/2013		Final Technical Report		05/01/2010 to 10/31/2011	
4. TITLE AND SUBTITLE Development of an Implantable Fish Spawning Sensor Tag				5a. CONTRACT NUMBER	
				N/A	
				5b. GRANT NUMBER	
				N00014-10-1-0798	
				5c. PROGRAM ELEMENT NUMBER	
				N/A	
6. AUTHOR(S) James Locascio David Mann Ernst Peebles				5d. PROJECT NUMBER	
				N/A	
				5e. TASK NUMBER	
				N/A	
				5f. WORK UNIT NUMBER	
				N/A	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of South Florida, College of Marine Science 140 Seventh Avenue, South St. Petersburg, FL 33701-5016				8. PERFORMING ORGANIZATION REPORT NUMBER	
				N/A	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Office of Naval Research 100 Alabama Street, SW Suite 4R15 Atlanta, GA 30303-3104				10. SPONSOR/MONITOR'S ACRONYM(S)	
				ONR	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
				N/A	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; Distribution is Unlimited.					
13. SUPPLEMENTARY NOTES Not Applicable					
14. ABSTRACT Information on the reproductive biology and spawning behavior of fishes is required for stock assessment and management of fisheries world-wide. Traditional methods for collecting these data are labor intensive and costly and consequently are often insufficient for many species. We propose to develop an implantable pressure sensor tag to record and transmit data on spawning activity and other biological functions important to fisheries management. The development of this sensor will rely in part on the integration of existing technologies produced by the biomedical and hi-tech industries.					
15. SUBJECT TERMS fish spawning; sensor tag					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			James Locascio; Dr. David Mann; Dr. E. Peebles
N/A	N/A	N/A	UU		19b. TELEPHONE NUMBER (Include area code)
					727-424-8680

20160222007

DISTRIBUTION STATEMENT A. Approved for public release; distribution is unlimited.
Development of an Implantable Fish Spawning Sensor Tag

Dr. James Locascio
College of Marine Science, University of South Florida
140 Seventh Avenue South, St. Petersburg, FL 33701
Phone: (727) 553-1193 Fax: (727) 553-1189 email: jlocasci@mail.usf.edu

Dr. David Mann
College of Marine Science, University of South Florida
140 Seventh Avenue South, St. Petersburg, FL 33701
Phone: (727) 553-1192 Fax: (727) 553-1189 email: dmann@marine.usf.edu

Mr. William Flannery (engineering subcontractor)
Spinnaker Electronics
451 43rd Ave. N.
Saint Petersburg, FL 33703

Grant Number: N000141010798
<http://www.marine.usf.edu>

PROJECT GOALS

The goals of this project were to develop an implantable tag to measure, record, and transmit data on fish spawning events. The design was intended to include input sensors to detect egg release from the body and motion sensors to measure changes in swimming speed and direction. The tag's purpose is to acquire more accurate data on the reproductive output of fishes, including measurements of variability between individuals in spawning frequency and magnitude over extended time periods (e.g. a spawning season). No such methods for directly measuring these events currently exists on the scale proposed by the development of this tag. Current methods rely on proxy data (e.g. back-calculated ages of eggs/larvae, gonad histology, and passive acoustics), which provide only a general estimate of egg production for use in stock assessment models and to understand variability associated with the stock-recruitment relationship. The goal of this project was to creatively apply technology to improve the quantity and quality of data available for studying the reproductive output of fishes. Field use of the tag was intended to be similar to currently used acoustic tags which are surgically implanted in the body cavity, with the addition of sensor placement near the ovipore to detect egg release.

OBJECTIVES

The objective of this project was to create an implantable data recorder to document spawning events and thereby improve our knowledge of reproductive behavior and output through direct measurements of wild fishes. The research value of this to fisheries scientists and managers is that direct, repeated measurements of spawning activity over extended periods (an entire spawning season) would better inform models for production estimates on the population level. These data are only measured indirectly in wild fishes through gonad samples or collection of eggs and larvae (e.g proxy measurements). In the case of spawning frequency and batch fecundity data, frequent harvesting of fishes and histology of gonads are required. Because of the time and expense associated with collection of these data they are often unavailable or insufficient for many exploited fish species, yet are required

for population models and stock-recruitment studies. The proposed spawning tag would greatly improve the quantity and quality of data available for understanding variability in the reproductive potential/output and in the stock-recruitment relationship of managed fish populations.

APPROACH

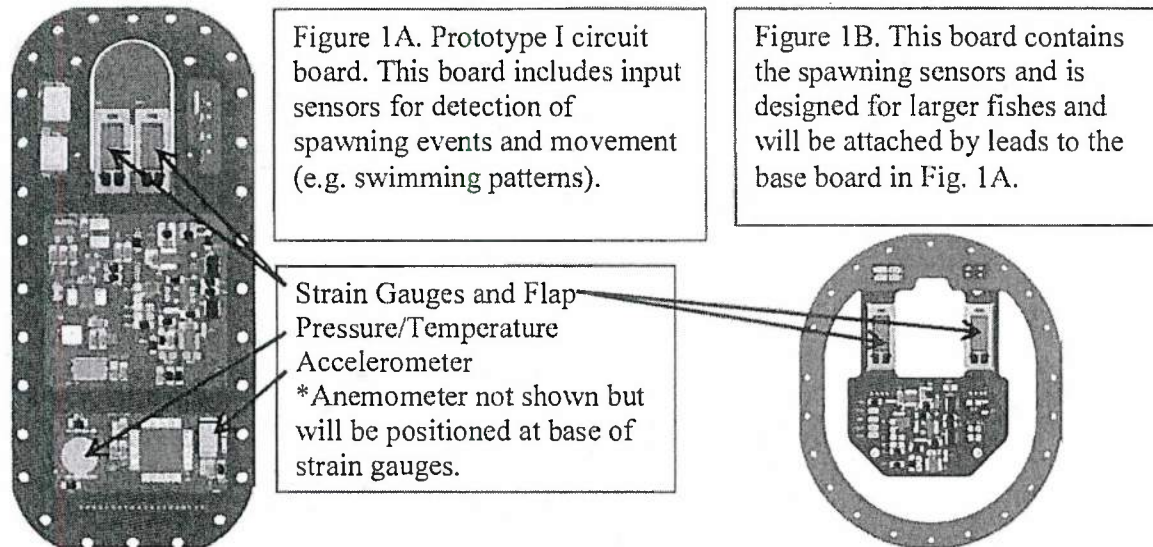
The original concept for the design of this tag was based on field observations at spawning aggregation sites of fish with greatly distended abdomens. These observations inspired our initial technical approach to document fish spawning events based on intra-ovarian pressure measurements made before and after spawning. A decrease in intra-ovarian pressure was expected following egg release.

Pressure measurements were made on captive spawning redfish (Port Manatee Hatchery Facility, Port Manatee, Florida) using a Millar Instruments pressure catheter inserted a fixed distance (15cm) into the ovary before and after spawning occurred. The initial results demonstrated that the pressure differential was very small, on the order of a pressure change that could have resulted from only a few inches of change in water depth. This small pressure difference would create a poor signal-to-noise ratio (signal=intraovarian pressure, noise=water depth) and demonstrated that this approach may be unreliable for documenting spawning. This result required us to consider a new approach for documenting fish spawning events.

Alternative input sensors were identified to detect swimming patterns and spawning events and the circuitry and board layout for the prototype were designed and built (Figure 1A and 1B). The sensor recording board uses an open source microcontroller system (Arduino) for rapid prototype development. A functional prototype was built with a recording accelerometer, magnetometer, and pressure sensor that save data to a microSD flash card (up to 32GB capacity). For engineering development two different sensor types were selected to measure egg flow: a custom designed hot-film anemometer (Tao Systems, Inc.; <http://www.taosystem.com/products/senflec/>) and a micro-measurement strain gauge (<http://www.vishaypg.com/micro-measurements/>).

Strain Gauge

A strain gauge measures stretch. These were fitted to the surface of a thin, flexible flap designed to be positioned over the ovipore and forced open by expulsion of eggs from the body during spawning. As the flap is bent during egg release the strain gauges become stretched and register a signal voltage associated with the spawning event. The magnitude of the spawning event would be calibrated from experiments conducted at hatchery facilities where eggs are collected in flow through filtration systems. The base of the flap is oriented in an anterior-posterior direction so it will not be forced open during swimming and is supported by a frame which allows form fitting to the body of the fish and an opening to avoid blockage by other excrement released from the body.



Figures: 1A and 1B Spawning tag prototype circuit board containing inertial motion sensors and flow sensors. The board is approximately 7x3 cm. The holes along the edges of the board are used for mounting to the ventral side of the fish. Initial tests will use sutures to secure the tag to the fish. In the final design, sutures that degrade in seawater would be used so that the tag will fall off after a certain amount of time.

Hot-Film Anemometer

Anemometer technology is used for measuring temperature and flow features in a variety of applications from industrial to biological. The custom hot-film anemometer selected is a flexible sensor fitted to the tip of a 2 mm diameter stainless steel wire bracket mounted on the circuit board and inserted just inside the oviduct. A small supply voltage (μV) is used to heat the sensor elements. During a spawning event heat is lost from the sensor element due to the flow of eggs over the surface of the sensor thus generating a signal associated with spawning. A prototype of this sensor is illustrated in Figure 2. The sensor is designed so that its function is independent of ambient temperature.

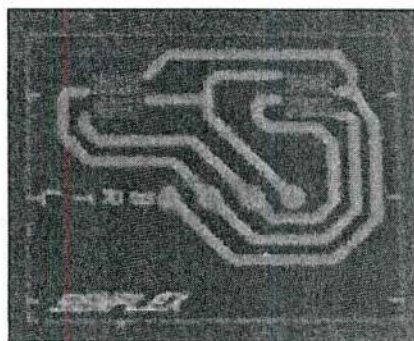


Figure 2. Custom hot-film anemometer sensor designed by Tao Systems, Inc. The sensor is 1x1.5 cm and flexible so it can be wrapped around a 2 mm stainless steel mounting tip for use in the oviduct of the fish to measure the flow of eggs.

Tag Potting

The electronic and battery portion of the tag is potted in permanent waterproof epoxy. The area of the tag with the microSD flash card and charge points for the battery is intended to be potted either with paraffin wax, or an RTV silicone that can be removed after deployment. The surface of the strain gauge sensors is covered with a thin layer of conformal silicone coating, which will protect them from

shorting, but still allow them to flex. Custom waterproof housings could be created as an alternative to potting material for early versions which will require removal of SD flash cards from the tag to recover data.

Battery Life

The spawning tag can be powered with a rechargeable prismatic (flat, like a cell-phone battery) lithium polymer battery. The battery for lab and field tests fits entirely beneath the main tag board. The battery provides 1.3 Ah at 3.7 Volts. Preliminary power draw tests demonstrate that during active data acquisition, power draw is about 10 mA. With continuous data acquisition, the tag would run for about five days, a period adequate for lab testing.

To enable longer duration deployments, low power modes may be used where the tag remains mostly in sleep mode and depth data are temporarily stored to memory onboard the microcontroller. For example, the tag will make a measurement of pressure and store that in internal memory and then go to sleep mode where the power draw is about 100 uA. If measurements are made for 10 ms once per second, the battery will last 100 x as long, or 500 days. The actual tag duration will be less than this, depending on the number of ascents detected when all of the sensors are turned on and sampled intensively.

The spawning tag also has a real-time clock that can be used to wake the tag from sleep mode. So, once it is generally known when a species spawns, the tag could be programmed to sleep when it is unlikely for spawning to occur. For example, if spawning is known to occur at dusk or early evening, the tag would spend more time in sleep mode during the day.

Tag Testing

Testing was planned to be performed in three phases. Phase I bench testing was used to demonstrate that the circuit functioned and the tag measured motion and water flow (as an analogue for egg release). A water flow system was used set to calibrate both sensors where the sensor output was measured as a function of flow rate. Once calibrated in a repeatable way, the system could also be calibrated by measuring the sensor output in response to the flow of spawned red drum eggs. This would allow us to determine whether the sensor calibration is different between eggs and water. Phase I was also used to verify that the epoxy potting of the tag allows it to perform underwater.

Phase II prototype tests were to be performed on live fish at the red drum aquaculture facility in Port Manatee, Florida (or similar aquaculture facility where spawning fishes are kept). This facility maintains a brood stock which spawns readily under controlled environmental conditions where the actual volume of spawned eggs can be measured. Data will be stored to onboard flash memory and results will be used for troubleshooting, overall performance evaluation, and prototype refinement.

In phase III the tag was intended to be field tested in wild fish. This phase was to be conducted in conjunction with a current NOAA MARFIN funded field study of goliath grouper by Chris Koenig (Florida State University) (Figure 3). In this study many goliath grouper (>30) have been acoustically tagged with Vemco transmitters and several recaptured at the same field sites off Jupiter, Florida. These fish appear to demonstrate high site fidelity at spawning aggregation sites.

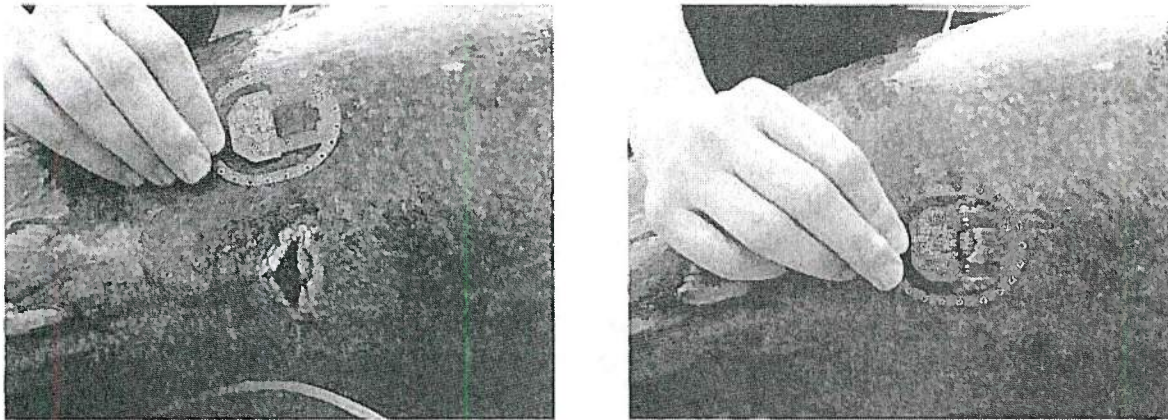


Figure 3. A spawning tag prototype circuit board held over the ovipore of an adult goliath grouper.

WORK COMPLETED

**Please refer to the Approach section for more details and context of work completed*

Intraovarian pressure differential measurements (before/after spawning) were made on three captive spawning redfish. The results indicated new methods for documenting spawning events should be considered. This was because while pressure changes were evident before and after spawning, they were small enough to be negated by slight changes of only a few centimeters in the fish's vertical position in the water column. New input sensors were identified along with inertial motion sensors to measure movement patterns of fishes (as previously described). Prototype circuit boards, (including support circuitry, input sensors, and micro SD data storage) and programming software were designed and built (Figure 1A and 1B). Initial bench tests of this prototype were completed and demonstrated the basic functionality of the programming software, circuitry and input sensors. Phase two tests were to be conducted on captive spawning redfish at the Port Manatee hatchery and phase three tests on goliath grouper in the field.

RESULTS

**Please refer to other sections for more details and context of results*

The results indicated that intraovarian pressure measurements are not a practical approach for documenting fish spawning events due to the confounding pressure changes caused by small vertical distances of only a few centimeters in the water column. Instead, a method is required which can target the release of eggs from the body while being immune to the influence of environmental conditions that may mask a signal associated with spawning. The expulsion of gametes from the body of a fish is a rapid event that should produce a high amplitude signal. The use of strain gauges, which measure 'stretch' should overcome the issues of pressure associated with position in the water column. Custom made and fitted strain gauges may be required in a later revision of this system after evaluation of test data in the field and lab. Both the strain gauge and hot film anemometer were functioned as planned in laboratory bench tests. Phase two and three tests were not completed due to logistical difficulties. Future research should focus on controlled experiments in a laboratory facility (e.g. hatchery) with a routine availability to fishes which spawn readily and which are resilient to handling and minor surgeries. Redfish (*Sciaenops ocellatus*) are good candidates for this but a separate broodstock must be maintained for experimental purposes so routine access is available for testing sensors and prototypes.

IMPACT/APPLICATIONS

The application of the spawning tag is to improve knowledge of the timing, location, and level of reproductive output of fishes. A device like this will provide more accurate data including measurements of variability among individuals in spawning frequency and magnitude over extended time periods (e.g. a spawning season). These data will be especially useful towards an improved understanding of the reproductive potential and output of spawning fish populations and in explaining variability associated with the stock-recruitment relationship and as such these data have direct benefit to the management of fisheries resources.